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Recent evidence of the presence of ERα and ERβ messages in prostatic tissues has appeared recently. Evidence suggested that Estrogen Receptor beta (ERβ) is down regulated during the precancerous prostate intraepithelium neoplasia (PIN) and reappear during the metastatic PCα. The applicant has proposed to synthesized novel selective ERβ agonist based on the lead structure 17βEstradiol, the Estrogen Receptor endogeneous ligand. The applicant ahs successfully synthesized the first generation of compounds with various aromatic moieties next to the 17α-vinyl of estradiol. 1H NMR studies show promising results that different aromatic moieties have different electronics influence on the vinyl proton signals which could suggest the selectivity toward ERα or ERβ. These results will be confirmed by biological assay which is in progress.

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Introduction

Prostate cancer is the most common form of cancer, other than skin cancer, among men in the United States. Prostate cancer often has no symptoms, however, if prostate cancer is found early, it can often be cured. A model of prostatic carcinogenesis has been proposed based on the morphologic continuum of prostatic intraepithelial neoplasia (PIN) and the multi-step theory of carcinogenesis progressing from normal prostatic epithelium through increasing grades of PIN to early invasive prostate carcinoma (PCa) (Figure 1). (http://www.bostwicklaboratories.com/edresour/pin/article.htm)

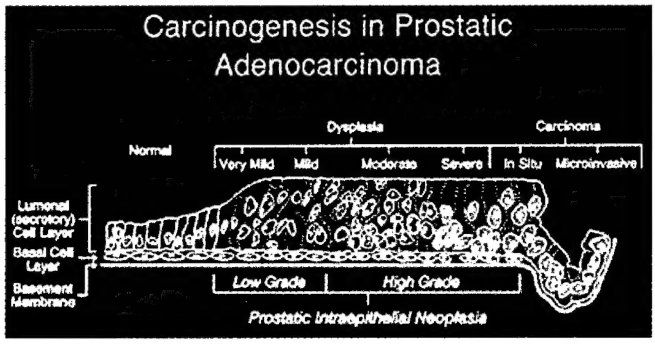


Figure 1

Prostate cancer development is initially steroid hormone dependent. While most studies have focused on the androgen receptor, recent evidence suggests that Estrogen Receptor beta (ERβ) is down regulated during the precancerous prostate intraepithelium neoplasia and reappears again during the metastatic prostate cancer. (Tsurusaki, T., 2003; Fixemer, T.,. 2003; Sasaki, M., 2002). Based upon these observations we hypothesized that selective ERβ agonist may play a role in the therapy of prostate cancer.

We have synthesized novel ERβ agonists based on the lead structure 17β estradiol, the endogenous ligand for both ERα and ERβ. The modification of the lead structure is based on the biological data and molecular docking studies. The biological data suggested that 17α-phenylvinyl estradiols were active against the prostate cancer DU-145 cell line. Molecular docking demonstrates that estradiol moiety fits in the pocket of ligand binding site while the phenyl moiety is adjacent to isoleucine 373 in ERβ or methionine 421 in ERα. Therefore, the selectivity toward ERβ could be modified by varying the aromatic moieties next to the 17α vinyl.

Body

This research project contains 4 specific aims. **The specific aim 1** is to synthesize the first generation of lead compound 17β estradiol (8 compounds) with series of aromatic moiety next to the 17α-vinyl group (phenyl; 1-, and 2-naphthalene; 2-, 3-, and 4-pyridine; 2-, and 3-thiophene). **The specific aim 2** is to submit the 8 compounds in the first generation to Professor Ho's laboratory in order to determine the selectivity toward ERβ or ERα and the inhibition of cell growth. The results from these experiments will guide for the second generation. (month 6-12). **The specific aim 3** is to select the compounds that give the highest selectivity to ERβ and introduce substituents onto the aromatic moiety at one or more positions (at least 15 new compounds will be synthesized). The substituents that will be introduced are hydrophobic side chain (methyl, ethyl, isopropyl), electron rich substituents (-OH, -OMe, -OEt), and electron deficient substituents (CF_3, amide linkage) (month 9-24). **The specific aim 4** is to submit new compounds prepared in the specific aim 3 to Professor Ho's laboratory for biological evaluation. At this point, the applicant will be able to generate sufficient data on the series of compounds to make a rational selection of potent and selective candidate for prostate cancer therapy (month 12-24).

The applicant has synthesized compounds in specific aim1 and the results has been presented in the National Meeting of the American Chemical Society, March 2004. The synthetic scheme is shown in Figure 2

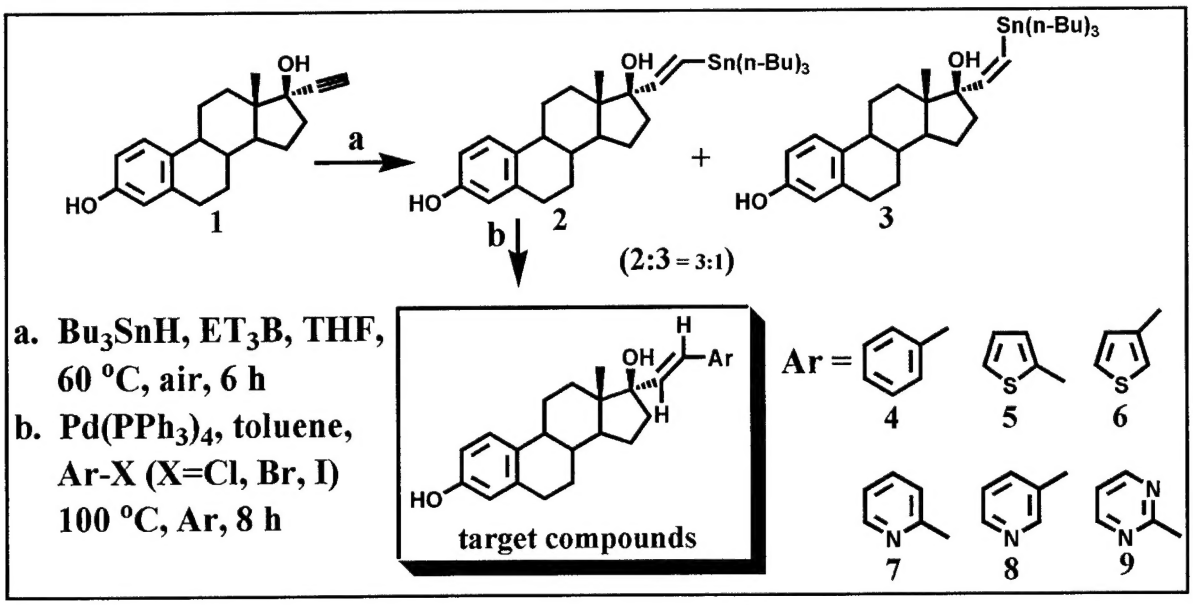
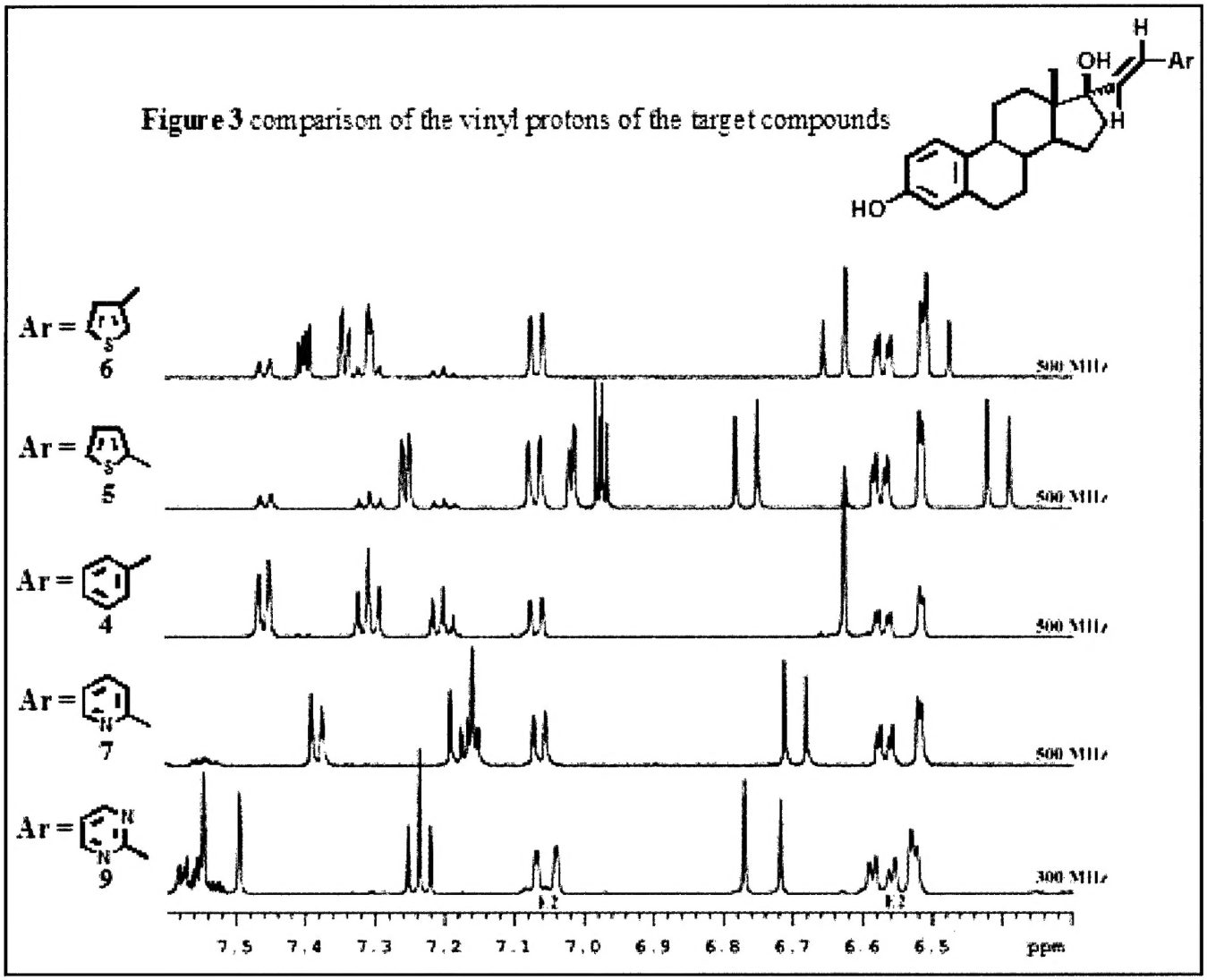


Figure 2 Synthetic scheme

Hydrostannation of compound **1** gave a mixture of *E*-, and *Z*- tributyltin vinylestradiol (compound **2**, and **3**) which was successfully separated by flash silica gel column chromatography. Stille coupling of ***E*-2** with aryl halide yielded the target compounds. The applicant has successfully optimized the conditions for the synthesis. These reactions are sensitive to oxygen, therefore it is necessary to carry out the reaction under argon atmosphere. Under careful controlled condition, the applicant were able to obtain the target compounds in high yield (60%-quantitative) with little amount of by-products. With the aid of automatic flash column chromatography, the applicant was able to purify the target compounds. Stille coupling reactions went well with aryl iodide, including those with strong electron withdrawing aromatic moiety such as 2-bromopyridine and 2-chloropyrimidine. All the target compounds were characterized by ^{1}H, ^{13}C NMR, and elemental analyses.

Interestingly, the proton NMR spectra of the target compounds show that the vinyl signals of aryl vinyl estradiol **5**, and **6** (electron donating aryl) appear at higher field than the vinyl signals of aryl vinyl estradiol **4**, **7**, **8**, and **9** (electron withdrawing aryl) (Figure 3).



These results suggested that different aromatic moieties next to the 17α-vinyl have different electronic influence on the vinyl protons. It can also suggested that these aromatic moieties could interact differently with isoleucine 373 in ERβ or methionine 421 in ERα which could lead to the selectivity toward ERβ or ERα. However, the biological data will support this hypothesis and we are in the process of submitting these compounds for the biological assay.

Key Research Accomplishments

We have completed the specific aim 1 and undertaken the specific aim 2 (in progress).

Reportable Outcome

These results have been presented at The 227th National Meeting of the American Chemical Society in Anaheim, CA, March 28 – April 1, 2004, poster number 233.

Conclusions

The first series of target compounds with varying the aromaties moieties next to the 17α-vinyl moiety have been synthesized and fully characterized. The ^{1}H NMR data suggested promising results that different aromatic moieties have different electronic effects on the vinyl proton signals which analogically suggest that these aromatic moieties could demonstrate selectivity toward ERb or ERα. These target compounds will be sending out for the biological assay as soon as possible.

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